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MANUFACTURING OF FISH SAUCE BY PROTEOLYTIC ENZYMES

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Different fish samples : sardine, macaroni, bourri, bolti and shark were used for the preparation of fish sauce. The applied proteolytic enzymes were: papain, trypsin, ficin and bromelain. Ripening periods were extended for 180 days at 37°. Changes in pH values, total soluble nitrogen (TSN), amino nitrogen (AN), volatile basic nitrogen (VBN), volatile organic acids (VOA) and in the free amino acid during fermentation period were considered in sardine; while the other fish samples were analyzed only before and by the end of fermentation period. The statistial analyses were used for comparing between the obtained results. the available data assured in the production of fish sauce rather than the other investigated enzymes. Such conclusion was based on regression analysis from which the added enzymes could be ascendingly ordered with respect to amino nitrogen of sardine sauce as follows: papain, R^2=0.725; trypsin, R^2 = 0.7629; bromelain, R^2=0.8219 and ficin, R^2=0.8975.

Key words: Fish sauce, Proteolytic enzyme, Sardine.

Introduction

Fish sauce, a widely used product in Southeast Asia, is a prolonged fermentation product of fish in high concentration of salt (greater than 20%).

Ooshiro *et al.* [1] used Sardine, mackerel and ami for making fish sauce by adding commerical enzymes; papain bromelain and trypsin. Fermentation temperature was varied; i.e. room temperature, 37° or 50°C and the initial pH (5.2) was adjusted to 4.5 and 6.5. Salt was added either 25% (based on fish weight) or 5 and 15% of the salt of first day and the remaining after 24 hr. Samples were minced by using handmincer; the weight of papain varied as 0.2, 0.3 or 0.5% out of fish weight and fermentation was proeeded to 340-350 days. Total soluble nitrogen, amino-type nitrogen, volatile base nitrogen, total volatile acid and individual volatile organic acids were determined during fermentation periods. Papain (0.3% out of fish weight) was satisfactory for proteolysis of the unminced sardines under the processing conditions of fermentation; 37°C, natural initial pH and 25% salt which as added at once together with the enzyme. The taste and colour of papain-added sample was satisfactory; while, the aorma was detected in matured control sample it was nto detected in the matured sample while it was not dectected in the matured samples containing the enzyme.

Taing *et al* [2] accelerated the production of fish cut into 2-3 cm pieces, uneviscerated and well mixed with 50g of salt. *Bacillus* C_1 and C_4 isolated from chinese fish sauce and strain No. 3-19-1 which isolated from sand of Kinko Bay were cultured in Sehgal and Gibbons Complex (SGC), medium with 3M NaCl and pH 6.6 - 6.8. To the control samples, (without bactria) 12.5 ml of solution containing NaCl and $MgSO_4$, the concentration of which were idential with the cultural medium was added. *Bacillus* C_1 showed remarkable effect on accelerating the process and the liquefaction step could be finished within 3 months. Ripening at various conditions was investigated and it was observed that volatile organic acids appeared within one month in the sample ripened at 30° under reciprocal shaking. Free amino acids of fish samples were determined and it was recognized that there was no great difference in free amino acid profile of control and that of bacteria-added samples.

This study reprots the preparation of fish sauce from selected fish samples, i.e. sardine, macaroni, bolti, bourri and shark. Changes that occur during the ripening periods were also determined.

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Materials and Methods

Fish samples. Sardines were purchased from Domiate Governorate; macaroni, bolti, bourri and shark were bought from the local market of Cairo Governorate while the shark was obtained from Suze Governorate.

Enzyme sources. Papain (2.5 units/mg solid), ficin (0.51 units/mg solid) and bromelain (1190 units/g solid) were purchased from Sigma Company While the trpsin (3.5 units/mg purchased from Sigma Company while the trypsin (3.5 units/mg solid) enzyme was obtained from Merck Company.

Salt. Sodium chloride of El-Nasr Company, Alexandria Governorate was used through manufacturing of the investi-

gated fish sauce.

Technological Methods. Fish sauce production. Sardine samples that used as a control were divided individually into four portions through which evisceration and beheaded were achieved. Other samples; i.e. macaroni, bolti, bourri and shark were treated similarly and cut into pieces approximately equal to the size of sardine pieces. Sodium chloride of 25% (based on fish weight) was added at once together with 0.3% of the responsed enzymes; i.e. papain, trypsin, ficin and bromelain. Fermentation was carried out in special glass jars, capped with screw-type plastic cover at a temperature of 37°C. Cysteine (110 mg) was added to the samples processed with papain or bromelain as recommended by Ooshiro *at al.* [1]. Fermentation was continued for 180 days through which a sample of the control (sardine) was analyzed monthly; while the other investigated fish samples were analyzed only by the end of fermentation period that extended to 180 days.

Analytical methods. Fish sauce samples were filterated and their chemical analysis were carried out in duplicates. pH, total soluble nitrogen (TSN), amino nitrogen (AN), volatile basic nitrogen (VBN) and volatile organic acids (VOA) of the prepared fish sauce samples were determined according to Ooshiro *et al* [1].

Determination of free amino acids. Protein and polypeptides in the sauce samples were precipitated by making 5 dilutions with 0.5% sulfosalicylic acid solution. The precipitate was seperated by centrifuging at 5000 rpm for 15 min. Further dilution (to 500 times) was carried out by using 0.02 N NaCl and the diluted sample was injected in the Beckman 118 GL amino acid analyzer available at the National Research Centre [2].

Statistical analysis. The SAS computer program was applied for carrying out regression analysis, correlation coeffecient and standard error of the results according to Helwig [3]. The 286 PC/AT 80286 Computer available at the expiry date project, Faculty of Agriculture, Ain Shams University, was used in such cases.

TABLE 1. RELATION BETWEEN TYPE OF PROTEOLYTIC ENZYMES AND CHANGES IN PH VALUES OF SARDINE SAUCE DURING FERMENTATION PERIODS.

Days of fermentation	pH Values of the sardine sauce of the				
	Control (without enzyme)	Other sauce sample containing proteolytic enzymes			
		Papain	Trypsin	Ficin	Bromelain
0	5.50	5.49	5.49	5.48	5.49
30	5.55	5.59	5.58	5.59	5.58
60	5.60	5.65	5.60	5.65	5.60
90	5.62	5.63	5.63	5.63	5.64
120	5.63	5.65	5.64	5.65	5.64
150	5.64	5.66	5.65	5.65	5.65
180	5.64	5.67	5.65	5.67	5.66

Results and Discussion

Table 1 shows the pH values of the control and the other fish samples which contain proteases enzymes; i.e. papain, trypsin, ficin and bromelain. During the period of fermentation which extended to 6 months (180 days), the pH value changed slightly from 5.5 to 5.64 in the control sample; and from 5.48 to 5.67 in the other sardine sauce samples containing proteolytic enzymes. These results are in agreement with those of Ooshiro *et al.* [1], who stated that pH of fish sauce samples prepared from japanese fish, i.e. sardine and mackerel species varied slightly, between 5.0 and 5.5; a value which was the critical pH for the activity of the responsed halophilic bacteria. However Taing *et al.* [4] proved that the pH did not change markedly during the production of fish sauce by using halophilic bacteria. Chayovan *et al.* [5] found that the pH of fish sauce from flounder (lean fish) and trout (fatty fish) ranged between 5.0-6.1 and 4.9-5.6, respectively. The pH of both sauce reached their highest levels at the sixth month of fermentation, similar to the results obtained in this study.

With respect to the changes that occur in the total soluble nitrogen, the data (Table 2) indicated that within the first month of fermentation, the total soluble nitrogen increased in both the control and other sardine sauces prepared using the poteolytic enzymes. Such increment was more pronounced at the ripening period of more than 120 days. At this period, the total soluble nitrogen of the control decreased slightly while that of the samples containing the enzymes showed

TABLE 2. RELATION BETWEEN TYPE OF PROTEOLYTIC ENZYMES AND CHANGES IN TOTAL SOLUBLE NITROGEN OF SARDINE SAUCE DURING FERMENTATION PERIODS.

Days of fermentation	Total soluble nitrogen (mg/ml) of the				
	Control (without enzyme)	Other sauce sample containing protelytic enzymes			
		Papain	Trypsin	Ficin	Bromelain
0	10.03	12.01	11.01	12.03	11.02
30	21.02	25.03	24.03	25.01	23.01
60	21.03	23.04	22.01	23.03	22.03
90	22.04	24.04	23.02	23.01	23.03
120	23.01	26.03	24.01	25.02	24.01
150	22.04	27.02	25.01	26.01	25.03
180	21.03	28.01	26.01	27.02	26.01
	Statistical measurements				
Intercept	15.5561	19.3163	17.0876	19.0876	0.6791
Slope	0.0477	0.0538	0.0512	0.0488	0.0065
Correlation coefficient	0.6960	0.7074	0.7118	0.6826	0.9285
Standard error	3.4897	3.8073	3.5823	3.6984	0.1835
F.Ratio	5.6369	6.0110	6.1627	5.2356	37.5134

upward trend until the end of fermentation. This indicates that the total soluble nitrogen content (TSN) of all sardine sauces produced in the presence of proteolytic enzymes was higher than the control at any given period of fermentation; statistical analysis given in the same table confirmed this trend. This may be due to the presence of proteolytic enzymes in test samples. The same pattern of changes was observed for amino nitrogen (AN) and volatile basic nitrogen (VBN). The results are in agreement with those reported by Filsinger *et al.* [6].

For instance, the amino nitrogen content of the samples showed a continous increase with the extension of fermentation periods as seen in Table 3. Slope of changes assured the previous conclusion. On the other hand, the volatile basic nitrogen correlated proportionally with the storage period; but the rate of change differed within different sardine sauces especially those containing proteolytic enzymes (Table 4). Dougan *et al.* [7] mentioned that (VBN) is considered as one of the remarkers of fish sauce aroma. He further mentioned that the activity of the added enzymes (papain, trypsin and bromelain) increased sharply during early few days of fermentation, afterwards the activity became weak due to high salt concentration.

With respect to the responsed properties of the sauce samples prepared from differnt fish soures; i.e. sardine, macaroni, bolti, bourri and shark, the data (Table 5) indicated that pH values differed within the tested samples, being higher in the samples manufactured with papain. However, there was no indication that the quality of fish sauce values varies significantly as a result of small variations in pH values [8,9].

TABLE 3. RELATION BETWEEN THE TYPE OF PROTEOLYTIC ENZYMES AND CHANGES IN AMINO NITROGEN CONTENT IN SARDINE SAUCE DURING FERMENTATION PERIODS.

Days of fermentation	Amino nitrogen content (mg/ml) of the Control (without enzyme)	Other sauce sample containing protelytic enzymes Papain	Trypsin	Ficin	Bromelain
0	3.03	4.01	4.02	5.03	5.03
30	13.01	15.04	14.02	15.02	15.04
60	12.03	14.02	13.03	14.01	14.03
90	14.04	16.01	16.03	17.03	16.04
120	15.03	16.01	16.02	17.01	17.03
150	14.02	17.02	17.01	18.02	18.02
180	14.03	18.01	17.02	19.03	19.01
	Statistical measurements				
Intecept	9.0719	10.3765	9.7715	0.5872	10.2376
Slope	0.0382	0.0483	0.0496	0.0076	0.0548
Correlatione coefficient	0.6536	0.0725	0.7629	0.8975	0.8219
Standard error	3.1333	3.2477	2.9797	0.2754	2.6889
F. Ratio	4.4756	6.6641	8.3547	24.8574	12.4927

The concentration total soluble nitrogen, amino nitrogen and the volatile basic nitrogen was also higher in the sample containing papain enzyme, a trend which related to the break down of protein during fermentation period. Saisithi *et al.* [10] came to the same conclusion. The total volatile acids of the same sample assured the previous trend; i.e. their values being higher in the fish sauce containing papain. These results confirm the view that in the early period of fermentation, the chemical reaction is probably more important for protein solubilization than the activity of enzymes. On the other hand, the responsed enzymes that are the dominant solubilizing agents during long term of fermentation, may cause the development of volatile compounds (volatile acids and volatile basic nitrogen) through ageing. However, it is well known that both of the volatile acids and bases develop very quickly in unpreserved fish [11,12].

The free amino acids showed a very pronounced variation within the investigated samples. For instance, glutamic acid having a value of 100 μ mole/ml in the macaroni fish sauce while it was 66.3 μ mole/ml in bourri fish sauce sample and both these values are higher than control (34.6 μ mole/ml) (Table 6). The amounts of sulphur containing amino acids (especially methionine) were also slightly higher in the fish sauce samples prepared with papain than other samples. On contrary, concentrations of aromatic amino acids (particularly tyrosine) were lower in the former samples than the latter one. Similar findings were given by Raa *et al.* [13]. Variations in free amino acids may either be related with the velocity of papain enzyme in the tested samples or to microbial degradation during long periods of fermentation [14].

Similar conclusion was given by Quaglia *et al.* [15] who proved that alcalase and papain are the best enzymes for a good nitrogen recovery with the possibility of producing sardine hydrolysates with high protein content, high nutritional value and high solubility.

TABLE 4. RELATION BETWEEN THE TYPE OF PROTEOLYTIC ENZYMES AND VOLATILE BASIC NITROGEN IN SARDINE SAUCE DURING FERMENTATION PERIODS.

Days of fermentation	Volatile basic nitrogen (mg/ml) of the Control (without enzyme)	Other sauce sample containing protelytic enzymes Papain	Trypsin	Ficin	Bromelain
0	0.52	0.62	0.63	0.63	0.61
30	1.02	1.21	1.12	1.12	1.12
60	1.12	1.32	1.21	1.32	1.21
90	0.83	1.21	1.13	1.12	1.01
120	0.94	1.43	1.32	1.43	1.31
150	1.42	1.82	1.62	1.71	1.62
180	1.61	2.21	2.01	2.11	2.01

TABLE 5. RESPONSED PROPERTIES OF THE INVESTIGATED SAUCE SAMPLES PREPARED BY PAPAIN ENZYME FROM DIFFERENT FISH SOURCES.

Fish sauces prepared from	pH		T.S.N.(mg/ml)		A.N. (mg/ml)		V.B.N.(mg/ml)		V.O.A.	
	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme
Sardine	5.64	5.67	21.03	28.01	14.03	18.01	1.61	2.21	0.52	0.74
Macaroni	5.71	5.76	24.12	25.03	15.03	14.09	1.53	1.43	0.63	0.83
Bolti	5.52	5.58	20.02	22.05	7.02	6.05	1.72	1.82	0.44	0.52
Bourri	5.83	5.94	20.22	22.02	7.51	7.01	1.82	2.01	0.72	0.95
Shark	6.01	6.21	19.31	22.03	16.12	20.02	2.32	2.62	7.05	7.05

T.S.N.=Total soluble nitrogen. A.N.=Amino nitrogen content. V.B.N.=Volatile basic nitrogen. V.O.A. =Volatile organic acid (expressed as ml of 0.1 NNaOH titrated).

TABLE 6. FREE AMINO ACIDS OF THE INVESTIGATED SAUCE SAMPLES PREPARED BY PAPAIN ENZYME FROM DIFFERENT FISH SOURCES.

Amino acid (μ mole/ml)	Fish sauces prepared from				
	Sardine	Macaroni	Bolti	Bourri	Shark
Aspartic acid	40.12	46.20	39.40	38.40	50.20
Threonine	19.20	28.30	25.20	26.20	30.20
Serine	18.50	5.90	15.60	16.70	13.20
Glutamic acid	34.60	100.01	70.20	66.30	105.30
Proline	16.20	15.20	12.60	11.20	13.16
Glycine	46.50	60.31	50.42	49.69	62.60
Alanine	70.20	73.20	80.69	85.20	95.60
Cystine	202.30	180.93	170.30	190.30	210.20
Valine	40.10	40.20	44.60	45.20	39.60
Methionine	5.30	8.30	7.80	6.20	6.70
Isoleucine	20.60	15.30	18.20	16.30	19.60
Leucine	30.92	22.60	23.60	25.60	29.30
Tyrosine	2.31	1.60	1.92	1.95	2.03
Phenylalanine	7.80	2.02	3.40	4.20	5.30
Ammonia	60.20	128.93	90.30	85.20	160.60
Lysine	36.30	60.20	50.20	55.20	40.20
Histidine	70.02	6.10	5.20	6.30	7.90
Arginine	2.30	0.90	0.60	0.95	1.90

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